PRELIMINARY OBSERVATIONS ON AMINES AND NITROSAMINES IN NON-NORMAL HUMAN GASTRIC CONTENTS

L. LAKRITZ, A.E. WASSERMAN, R. GATES & A.M. SPINELLI

U.S. Department of Agriculture,
Eastern Regional Research Center,
Philadelphia, Pa., USA

INTRODUCTION

The formation of nitrosamines in the gastric contents of animals from ingested nitrite (or nitrate) and secondary amines has been demonstrated experimentally (Mysliwy et al., 1974; Sen et al., 1969). The presence of nitrosamines in human gastric contents is of interest because of the carcinogenic nature of most of the nitrosamines (Druckrey et al., 1967; Lijinsky & Greenblatt, 1972), but has been difficult to demonstrate. Sander & Seif (1969) gave diphenylamine and nitrate to 31 subjects and recovered *N*-nitrosodiphenylamine, a non-carcinogenic nitrosamine, in the gastric contents of 11 of the subjects. There was a positive correlation between the presence of the nitrosamine and the increase of stomach acidity in these patients. Sander (1973), however, swallowed piperidine and cured ham extract, which contained nitrite, and was unable to find *N*-nitrosopiperidine (NPIP) in gastric contents he recovered by induced vomiting. Two subjects, a smoker and a non-smoker, were fed a meal consisting of luncheon meat, egg and milk. NPIP was found at levels less than 1 µg/kg after a residence time of 30 or more minutes. Trace amounts of the nitrosamine were found more often in the gastric contents of the smoker than the non smoker (Walters, 1976).

We are reporting here preliminary results on the amines and nitrosamines found in stomach contents of 35 fasting patients, hospitalized for a variety of conditions and undergoing routine clinical gastric examination.

EXPERIMENTAL

Gastric contents of subjects fasting at least 8 hours prior to examination were removed by medical personnel, using routine aspiration through a Levin tube. Where appropriate, samples were taken before and after administration of histamine or pentagastrin to stimulate gastric secretion. The contents remaining after clinical study were frozen and stored at -20°C until they could be analysed for amines. Initially, the gastric samples for nitrosamine analyses were stored frozen. Subsequently, each sample was divided into two portions, the pH was determined and one portion was made alkaline by the addition of sodium hydroxide, to prevent possible nitrosamine formation during storage. Both fractions were stored frozen until analysed.

Volatile amines

The pH values of the gastric contents were determined and adjusted to pH 1-2, if necessary, with hydrochloric acid. The contents were then extracted twice with equal volumes of diethyl ether. The pooled ether extracts were washed twice with water and the water washes were added to the gastric sample. Residual ether was removed from the gastric samples by blowing a stream of nitrogen over them. The samples were then adjusted to pH 12 with sodium hydroxide and steam distilled. One and a half volumes of distillate were collected in cold traps (ice bath) containing 2 ml 2N hydrochloric acid and 8 ml water. This material was freeze-dried, redissolved in 1-2 ml water, adjusted to pH 10 with sodium hydroxide and analysed by gas chromatography. For histamine, a 183 cm x 2 mm I.D. glass column packed with Chromosorb 103 was used. The flow of the helium carrier gas was 50 ml/min. Injector and detector temperatures were 170°C and 260°C, respectively. The oven was heated isothermally at 105°C for 10 minutes and then programmed at 8°/min to 250°C. An alkali flame-ionization detector was used. Dimethylamine and trimethylamine were separated on a 183 cm x 2 mm I.D. glass column packed with Amine 220 (12%) plus potassium hydroxide (8%) on 100/120 mesh Chromosorb WAW. Carrier gas (nitrogen) flow was 17 ml/min. Injector and detector temperatures were 190°C and 220°C, respectively, and the column was heated isothermally at 60°C. Volatile amines were detected with the flame-ionization detector.

Non-volatile amines

The solution remaining in the pot after steam distillation contained the non-volatile amines. It was saturated with NaCl and extracted three times with equal volumes of a mixture of butanol:trichloromethane (1:1). The combined organic phase was re-extracted with 1.5 volumes of a mixture of heptane:0.2N hydrochloric acid (15:1). The aqueous layer, which contained the amines, was freeze-dried. For derivatization, the dried sample was dissolved in 1-2 ml distilled water and saturated with sodium carbonate. To this solution, 0.9 ml acetonitrile containing 3 mg 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD.Cl) was added and heated at 50°C for 30-40 minutes. The tube containing the

reaction mixture was frozen and the organic layer was removed. The NBD derivative of the amine was spotted on Silica Gel G plates and separated with a solvent system consisting of trichloromethane:methylene chloride (8:2). 5-Dimethylamino-1-naphthalene-sulfonyl chloride (Dansyl chloride) derivatives of the amines were also prepared, starting with 0.2 ml of the reconstituted freeze-dried material saturated with sodium bicarbonate. To this was added 1 ml reagent (40 mg Dansyl chloride in 10 ml acetone) and the mixture was shaken for 15-17 hours. After being dried under nitrogen, the residue was extracted three times with ethyl acetate. The combined extracts were concentrated to 1 ml; the concentrate was spotted on 250 μ Silica Gel G plates and separated by two-dimensional chromatography, using ethyl acetate: cyclohexane (65:35) in one direction and benzene: triethylamine (80:20) in the other. The plates were viewed under ultra violet light (366 nm) and the compounds were identified by the R_f values.

Nitrosamines

Ten grams sodium chloride and 1 ml of *N*-nitrosoethylmethylamine solution, an internal standard, were added to the sample of gastric contents, pH ≈ 10, and transferred to a liquid-liquid extraction apparatus. After continuous extraction with methylene chloride for 5 hours, the extract was dried with anhydrous sodium sulfate and concentrated to 4 ml in a Kuderna-Danish concentrator with a 3-bubble Snyder column, then to 1 ml with a micro-Snyder column in a 65°C water bath. The nitrosamines were separated by gas chromatography as described previously (Pensabene et al., 1974) and detected by a Thermal Energy Analyzer (Fine et al., 1975). All nitrosamine-positive samples were confirmed by mass spectrometry (Dupont Model 492).

Nitrite and nitrate

Gastric samples were analysed for nitrite by employing the procedure of Fiddler, 1977. Nitrates were analysed as nitrite, after being reduced by passing the samples through a cadmium column (Kamm et al., 1965).

RESULTS AND DISCUSSION

Gastric contents from 35 patients were analysed for the presence of nitrosamines. In addition, a number of samples were also taken following gastric stimulation with histamine or pentagastrin, making a total of 57 samples analysed. Nitrosamines [*N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA) or *N*-nitrosopyrrolidine (NPYR)] were found in 7 samples in concentrations ranging from 2 to 30 μg/kg.

The gastric contents of the first 9 patients, including patients 1, 3 and 4 in Table 1, were frozen after they were obtained. After the results of these analyses were seen, with NDEA concentrations ranging from 5 to 30 μg/kg, it was decided to make one portion of

each sample alkaline, to prevent the possibility of nitrosamine formation in the frozen state prior to analysis. There is no evidence that the three high values, 23, 26 and 30 µg/kg NDEA, were due to formation of the nitrosamine after the gastric material was frozen, but it is interesting to note that the remaining positive nitrosamine results were low, ranging from 2 to 6 µg/kg of either NPYR or NDMA. In the gastric contents of an additional 4 patients, nitrosamines at concentrations too low for mass spectrometric confirmation were noted. No nitrosamines were detected in the gastric contents of the remaining 25 patients.

Nitrite and nitrate analyses were carried out on a limited number of samples because the volume of gastric material was often insufficient to perform all the analyses. Twenty-two of the gastric samples, in which nitrosamines were absent, averaged 1.1 mg/kg nitrite and 23.7 mg/kg nitrate. Three samples, in which nitrosamines were detected, were analysed for nitrite and/or nitrate (Table 1). Two of the samples (on the same patient) contained large concentrations of nitrite, approximately 70 mg/kg.

Table 1. Nitrosamines found in human gastric contents - levels of nitrosamines, nitrite, nitrate, pH and diagnosis

Patient	Nitrosamine		NO_2	NO_3	pH	Diagnosis
	Compound	µg/kg	mg/kg	mg/kg		
1	NDEA	30	69		1.6	Duodenal ulcer
	NDEA[a]	26	76		1.5	
3	NDEA	5			2.2	Marginal ulcer
4	NDEA	23			2.9	Marginal ulcer & cirrhosis of liver
15	NPYR[a]	6			6.4-7.5	Atrophic gastritis
26	NDMA	2	0	26	2.9	Not known
31	NDMA[a]	2			1.9	Gastritis, duodenitis

[a] These samples were obtained following gastric secretion stimulation with histamine or pentagastrin.

The pH of the gastric samples, measured shortly after aspiration, ranged from 0.9 to 7.8, with most samples having values around pH 2.0. The pH of the gastric contents containing nitrosamines were similarly grouped around pH 2, but one sample had a pH of 6.4-7.5. There does

not appear to be a relationship between the nitrosamine content and the pH of the gastric samples.

This study was conducted on samples obtained from several hospital gastroenterology laboratories. As expected, the clinical diagnosis of the patients involved disorders of the gastrointestinal tract. A partial breakdown of the diagnoses obtained include: duodenal ulcer, 8: gastric ulcer, 1; peptic (unspecified) ulcer, 2; ulcers, 5; oesophagitis, duodenitis and gastritis, 4. Diabetes, cirrhosis and anemia were also noted. The types of conditions diagnosed in patients in whose gastric contents nitrosamines were found (Table 1) did not differ from those in patients in whom nitrosamines were absent. Due, perhaps, to the small scope of this study, no correlation could be observed between the clinical diagnosis and the presence of nitrosamines.

The presence of amines in gastric contents was studied in pooled gastric samples in order to obtain sufficient material for investigation; therefore only qualitative analyses were carried out. Volatile amines found included dimethylamine, trimethylamine and histamine. The non-volatile amines were cadaverine, putrescine, ethanolamine and tryptamine.

SUMMARY

Gastric contents from fasting humans were pooled and analysed for amines. Volatile amines present were dimethylamine, trimethylamine and histamine; non-volatile amines found were cadaverine, putrescine, ethanolamine and tryptamine. Gastric contents from 35 patients, some before and/or after gastric stimulation with histamine or pentagastrin, were analysed for nitrosamines. *N*-Nitrosodiethylamine (NDEA) (5-30 µg/kg) was present in four samples, *N*-nitrosodimethylamine (NDMA) (2 µg/kg) in two samples and *N*-nitrosopyrrolidine (NPYR) (6 µg/kg) in one sample. pH, nitrite and nitrate determinations were made on some samples. Medical diagnosis of patients could not be correlated with the presence of nitrosamines in the gastric contents.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the medical assistance of Leslie Bernstein, M.D., Division of Gastroenterology, Montefiore Hospital, Bronx, NY, and Franz Goldstein, M.D., Chief, Department of Gastroenterology, Lankenau Hospital, Philadelphia, Pa. The authors thank the National Institutes of Health, National Cancer Institute for providing a Thermal Energy Analyzer to our Laboratory on a loan basis under Contract NO1-CP-55715.

Reference to a brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

REFERENCES

Druckrey, H., Preussmann, R., Ivankovic, S. & Schmähl, D. (1967) Organotrope carcinogene Wirkungen bei 65 verschiedenen *N*-Nitroso-Verbindungen an BD-Ratten. *Z. Krebsforsch.*, *69*, 103-201

Fiddler, R.N. (1977) Collaborative study of modified AOAC method of analysis for nitrite in meat and meat products. *J. Assoc. Off. Anal. Chem.*, *60*, 594-599

Fine, D.H., Rufeh, F., Lieb, D. & Rounbehler, D.P. (1975) Description of the Thermal Energy Analyzer (TEA) for trace determination of volatile and nonvolatile *N*-nitroso compounds. *Anal. Chem.*, *47*, 1188-1190

Kamm, L., McKeown, G.G. & Smith, D.M. (1965) New colorimetric method for the determination of nitrite and nitrate content of baby foods. *J. Assoc. Off. Anal. Chem.*, *48*, 892-897

Lijinsky, W. & Greenblatt, M. (1972) Carcinogen dimethylamine produced *in vivo* from nitrite and aminopyrine. *Nature (Lond.)*, *236*, 177-178

Mysliwy, T.S., Wick, E.L., Archer, M.C., Shank, R.C. & Newberne, P.M. (1974) Formation of *N*-nitrosopyrrolidine in a dog's stomach. *Br. J. Cancer*, *30*, 279-283

Pensabene, J.W., Fiddler, W., Gates, R.A., Fagan, J.C. & Wasserman, A.E. (1974) Effect of frying and other cooking conditions on nitrosopyrrolidine formation in bacon. *J. Food Sci.*, *39*, 314-316

Sander, J. & Seif, F. (1969) Bakterielle Reduktion von Nitrat in Magen des Menschen als Ursache einer Nitrosamin - Bildung. *Arzneim. Forsch., 19*, 1091-1093

Sander, J. (1973) *Formation of nitroso compounds in humans: evaluation of the problem.* In: Tinbergen, B. & Krol, B.J., eds, *Proceedings of the International Symposium on Nitrite in Meat Products, Zeist,* Wageningen, Centre for Agricultural Publishing and Documentation, pp. 251-255

Sen, N.P., Smith, D.C. & Schwinghamer, L. (1969) Formation of *N*-nitrosamines from secondary amines and nitrite in human and animal gastric juice. *Food Cosmet. Toxicol., 7*, 301-307

Walters, C.L. (1976) *Nitrosation of food amines under stomach conditions.* In: Tinbergen, B. & Krol, B.J., eds, *Proceedings of the 2nd International Symposium on Nitrite in Cured Meat Products, Zeist*, Wageningen, Centre for Agricultural Publishing and Documentation, pp. 125-133